Investigations on the metabolic fate of prochloraz in soil under field and laboratory conditions

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Abstract: The degradation of prochloraz in different soils was investigated in field and laboratory experiments. In laboratory degradation experiments in the dark, initial prochloraz concentrations decreased to 30–64% within 56 days, depending on temperature and soil pH. In neutral to basic soils, formation of up to 3.7% of the metabolite prochloraz-urea was observed. The rate of mineralization was strongly pH-dependent, not exceeding 3.2% in the acidic and 18.3% in the neutral to basic soils. Amounts of non-extractable residues ranged from 14 to 31%. Under field conditions, prochloraz disappeared much more rapidly with DT_{50} values of 11–43 days. The metabolites prochloraz-formylurea and prochloraz-urea were found in significant concentrations. Laboratory experiments with fresh and sterilized soils under UV irradiation confirmed the enhancing effect of light on the formation of the primary metabolite, prochloraz-formylurea. The latter is hydrolysed to prochloraz-urea predominantly by microbial degradation.

Keywords: prochloraz; degradation; soil; UV irradiation; metabolites

1 INTRODUCTION

Prochloraz (*N*-propyl-*N*-[2-(2,4 6-trichlorophenoxy)-ethyl]imidazole-1-carboxamide; Fig 1, **1**) is applied as a protective and eradicant fungicide against *Pseudocercosporella, Pyrenophora, Rhynchosporium* and *Septoria* spp in cereals. Its mode of action is based on inhibition of sterol-14α-demethylase in fungal ergosterol biosynthesis.[1]

Despite the significance prochloraz has gained in agricultural practice since its introduction in 1977,[2] little has been published on its fate in soil. In one long-term field study, only the parent compound was determined regularly. Prochloraz residues were detectable in deeper soil layers (10–30 cm) over two vegetation periods. 2,4 6-Trichlorophenol, which was determined only occasionally, was proposed to be a major metabolite due to increased concentrations in plots treated with prochloraz as compared to those with no history of such treatment.[3] Within a toxicity study, the fate of prochloraz in sediments irradiated at 16-h light/8-h dark intervals was investigated. Depending on the organic matter content, small concentrations of prochloraz-formylurea (*N*′-formyl-*N*-propyl-*N*-[2-(2,4 6-trichlorophenoxy)ethyl]urea; **2**) were found, possibly as a result of photodegradation.[4]

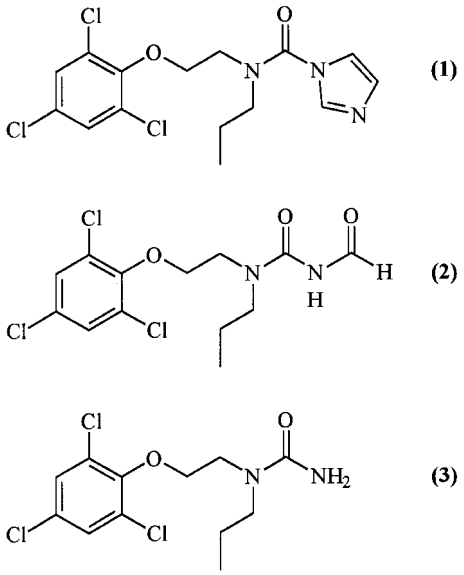

Figure 1. Chemical structures of (**1**) prochloraz and its metabolites (**2**) prochloraz-formylurea and (**3**) prochloraz-urea.

Experiments on degradation of prochloraz by environmental bacteria strains hinted at formation of the same compound during microbial metabolization.[5] The fate

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of prochloraz in mammalian organisms is well documented. Metabolism experiments with rats revealed that the main pathway of degradation starts with prochloraz-formylurea which is rapidly hydrolysed to prochloraz-urea (*N*-propyl-*N*-[2-(2,4 6-trichlorophenoxy)ethyl]urea; **3**). Ring hydroxylation also occurs, leading to some minor metabolites.[6,7] Since the compounds of the main metabolic pathway in rats are taken to be important in plants too, an analytical method was developed for the simultaneous determination of prochloraz and its metabolites after hydrolysis of these compounds to 2,4 6-trichlorophenol.[8]

The objective of this study was to investigate the residual behaviour of prochloraz in soil under different conditions. Laboratory degradation experiments were conducted with typical soils from Lower Saxony, Germany, differing in pH value, organic carbon content and texture. In order to set up detailed mass balances including mineralization and formation of non-extractable residues, these experiments were carried out with ^{14}C-labelled prochloraz. The results were compared with data from field studies conducted at two sites with different soil properties. The influence of light on the degradation of prochloraz was determined by laboratory experiments in a closed apparatus under UV irradiation.

2 MATERIALS AND METHODS

2.1 Chemicals

[*Phenyl-U*-^{14}C]prochloraz with a radiochemical purity of 97.8% and a specific activity of 1.473 MBq mg^{-1} was obtained from Campro Scientific, Emmerich, Germany. Non-labelled prochloraz (chemical purity >99%) came from Riedel-de Haën, Seelze, Germany, and the non-labelled metabolites prochloraz-formylurea and prochloraz-urea were supplied by AgrEvo, Saffron Walden, UK.

2.2 Investigation sites and soils

The investigations were conducted at two catchment areas of the German Research Society's Special Collaborative Program 179 'Water and Matter Dynamics in Agro-Ecosystems' in Lower Saxony, Germany. Field degradation experiments were carried out on a clayey silt soil (NK1) at Neuenkirchen in the foreland of the Harz mountains and on a silty sand soil (NW1) at Nienwohlde in the Lüneburger Heath. For the laboratory studies, a silty loam soil (NK2) from Neuenkirchen and three loamy sand soils (NW2, NW3, NW4) from Nienwohlde were additionally selected. Properties of all the soils are given in Table 1.

Table 1. Properties of the soils from the investigation sites Neuenkirchen (NK) and Nienwohlde (NW), Lower Saxony, Germany

Soil	*Sand (%)*	*Silt (%)*	*Clay (%)*	C_{org} *(%)*	*pH ($CaCl_2$)*
NK1	2	80	18	0.97	7.1
NK2	3	69	28	0.77	7.8
NW1	76	19	5	1.52	5.3
NW2	86	11	3	0.53	5.8
NW3	77	15	8	2.52	6.8
NW4	81	13	6	4.44	5.9

2.3 Field studies

Prochloraz was applied according to common agricultural practice as a 300 g litre^{-1} emulsifiable concentrate ('Sportak α'®, AgrEvo, Frankfurt, Germany), To avoid carry-over of prochloraz residues, different plots were chosen for treatment in April/May 1995 and 1996 at each site. Application rates were 210 g AI ha^{-1} and 300 g AI ha^{-1} in Neuenkirchen and 300 g AI ha^{-1} and 450 g AI ha^{-1} in Nienwohlde in 1995 and 1996, respectively. Control plots of 225–540 m^2 were left untreated each site and year. In 1996 at both sites, the crop stand was cut on four 1-m^2 plots just before fungicide treatment. This was done to increase the amount of prochloraz that reached the soil surface and in order to ensure detection of metabolites which were expected to be formed only in low concentrations. Two of these reaped plots were located inside the treated area and two inside the control plot. Soil samples were taken from the 0–5-cm layer. One bulked sample of about 1 kg weight consisted of four soil cores which were collected by means of a bulb planter. First sampling was conducted shortly after application, except for Neuenkirchen in 1996. Thereafter, samples were taken in successively increasing intervals, eg at 1, 2, 5, 7, 14, 28 and 56 days after application. In 1996, sampling was done on the initially cut plots. The field moist soil was sieved (<2 mm) and frozen at −20 °C until analysis.

2.4 Laboratory studies

2.4.1 Degradation experiments in the dark

To preserve soil inherent microbial activity, fresh soil samples were stored for no longer than one week before use in degradation experiments. Preliminary tests had proven that a sufficient activity level as measured by dehydrogenase activity according to Malkomes[9] was maintained over the incubation periods investigated.

In the first degradation experiment, clayey silt soil samples (<2 mm, 25 g) in 300-ml Erlenmeyer flasks were fortified with [^{14}C]prochloraz solution in methanol (3120 µg kg^{-1} soil) and mixed thoroughly. The amount applied was about six times the recommended application rate of 400–600 g AI ha^{-1} in cereals.[10] The initial activity was 41.3 kBq. After adjusting soil water content to 40% of the maximum water holding capacity,[11] the flasks were closed. According to the OECD guideline for testing of chemicals, the glass stoppers were equipped with two stopcocks and an internal vessel filled with potassium hydroxide solution (0.1 M; 8 ml) to absorb released [^{14}C]carbon dioxide.[12] Absorption solutions were changed weekly in order to determine mineralization rates by liquid scintillation counting (LSC). Soils were incubated in the dark at 25 °C. Eight soil samples were taken 1 h, 2, 7, 14, 28,

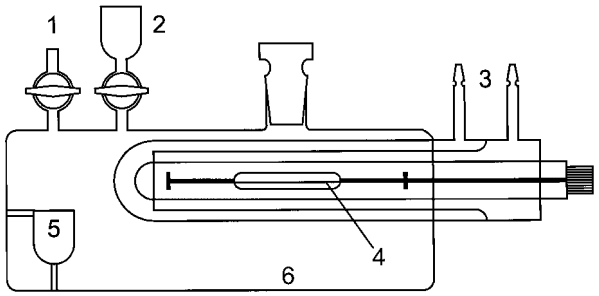

Figure 2. Apparatus for degradation experiments under UV irradiation. 1: inlet valve, 2: outlet valve with activated charcoal filter, 3: water cooling, 4: Hg medium-pressure lamp within Pyrex® cooling jacket, 5: vessel for potassium hydroxide absorption solution, 6: soil sample placed directly under the lamp in a 3–5-mm layer.

56, 112 and 300 days after prochloraz application and frozen at −20 °C until analysis.

All subsequent experiments were incubated at 30 °C. The amount of prochloraz was reduced to 1143 $\mu g\,kg^{-1}$ soil, corresponding to an initial radioactivity of 42.1 kBq. Three to five soil samples were taken, covering a period of 56 days.

For one experiment, the soil NK1 was amended with 1% of ground triticale straw and preincubated for six weeks at 30 °C in order to increase its inherent microbial activity. Water content after pre-incubation amounted to 60% of the maximum water holding capacity and was left at this level throughout the experiment.

To conduct experiments under sterile conditions, the soils used, all glassware and the potassium hydroxide solution for absorption of [^{14}C]carbon dioxide were sterilized three times for 15 min each with steam at 130 °C in an autoclave. In these experiments, one soil sample was taken in the middle and one at the end of a 56-day incubation period.

2.4.2 Degradation experiments under UV irradiation

A special apparatus was designed for irradiating soil samples during degradation experiments with [^{14}C]prochloraz (Fig 2). It consisted of a glass tube with two stopcocks, an internal vessel for potassium hydroxide absorption solution, one opening with a glass stopper and another opening for the UV lamp. The latter was an Hg medium-pressure lamp (TQ 150, Heraeus, Hanau, Germany) within a cooling jacket of Pyrex® glass (cut off at $\lambda = 290$ nm). It was connected to a timer clock to allow irradiation at intervals.

Soil samples (25 g, adjusted to 40% of maximum water-holding capacity) were placed into the tube and fortified as described above. They were incubated between 14 and 66 h with total irradiation periods ranging from 8 to 24 h. Irradiation took place in 4-h or 15-min intervals with subsequent dark periods of the same duration. Water losses through evaporation were compensated daily in the case of incubation periods exceeding 24 h. Thereafter, irradiated soil samples were treated in the same way as those from the experiments in the dark.

2.5 Analytical methods

2.5.1 Residue analysis

Soil samples were analysed according to the principles of the DFG S19 multi method.[13] Extraction of soil (50 g dry weight) with acetone (100 ml) and water (50-X ml; X = soil inherent water content) was followed by filtration using a Büchner funnel and liquid–liquid partitioning with cyclohexane (100 ml). The organic phase was dried over anhydrous sodium sulfate, evaporated and redissolved in ethyl acetate + cyclohexane (1 + 1 by volume). After gel permeation chromatography and solvent evaporation, samples were redissolved in 2-propanol (1 ml).

Residues were determined by high-performance liquid chromatography (HPLC) using an HP 1050 system with an HP 1040A diode array detector (Hewlett-Packard, Avondale, USA). The column was an RP-select B 125-4 (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile and buffered water (0.001 M potassium dihydrogenphosphate, adjusted to pH 8 by addition of potassium hydroxide). The following gradient was applied: 0 min–30% acetonitrile, 2 min–30% acetonitrile, 20 min–66% acetonitrile. Detection wavelengths were 205 nm and 225 nm. The method was validated by conducting fortification experiments with soil. For each substance, a concentration of 10 $\mu g\,kg^{-1}$ was found to represent the lower limit of the practical working range.[14] Soil samples treated with prochloraz were analysed in duplicate. One control sample of the respective sampling date was always analysed in parallel.

2.5.2 Radiotracer analysis

Aliquots of the potassium hydroxide solutions used for the absorption of [^{14}C]carbon dioxide were put into LSC vials and filled up with scintillation cocktail Quicksafe A (10 ml; Zinsser, Frankfurt, Germany). Radioactivity was measured using a Tri-Carb 2500 liquid scintillation counter (Packard, Meriden, USA).

Soil samples (25 g) from degradation experiments were extracted as described above. After filtration, liquid–liquid partitioning and drying, the extract was redissolved in ethyl acetate (2 ml). Aliquots (50 + 100 µl) in LSC vials were counted with Quicksafe N scintillation cocktail (5 ml; Zinsser).

To determine the amounts of non-extractable residues, extracted soil samples were thoroughly dried and four aliquots (0.2 g) of each soil were combusted in an Ox-500 oxidizer (Harvey, Hillsdale, USA). The released [^{14}C]carbon dioxide was absorbed in Carbomax Plus scintillation cocktail (Packard).

The extracts from degradation experiments were examined by thin-layer chromatography (TLC). Silica gel plates SILGUR-25 ($20 \times 20\,cm^2$) with pre-concentration zone (Macherey-Nagel, Düren, Germany) were developed in either toluene + ethanol (15 + 1 by volume) or hexane + ethyl acetate (3 + 1 by volume). TLC chromatograms were recorded using a Trace-

master 20 TLC scanner (Berthold, Munich, Germany).

3 RESULTS

3.1 Residue dynamics in the soils NW1 and NK1

3.1.1 Field experiments

In 1995 and 1996, prochloraz concentrations dropped markedly within a period of 56 days after application at both experimental sites. In the clayey silt soil NK1, they decreased from 116 to 28 $\mu g kg^{-1}$ in 1995. In the following year, a decline from 187 to 42 $\mu g kg^{-1}$ was observed, the higher initial concentration being due to direct soil application. In the silty sand soil NW1, prochloraz concentrations decreased from 51 to 13 $\mu g kg^{-1}$ in 1995 and from 232 to 103 $\mu g kg^{-1}$ after direct soil application in 1996. The fungicide's field degradation rate was determined by calculation of DT_{50} values according to Timme *et al.*[15] For NW1, they were 11 days in 1995 and 43 days in 1996, whereas for NK1 identical values of 13 days were found in each year.

Concentration curves of the metabolites prochloraz-formylurea and prochloraz-urea showed a characteristic pattern in each experiment. Concentrations of prochloraz-formylurea increased quickly during the first days after application, reaching maxima between the third and the fourteenth day. In 1995, these maximum values were 30 $\mu g kg^{-1}$ and 69 $\mu g kg^{-1}$ and in 1996 they amounted to 58 $\mu g kg^{-1}$ and 67 $\mu g kg^{-1}$ in NK1 and NW1, respectively. Thereafter, concentrations dropped, although prochloraz-formylurea remained detectable even after 56 days. Curves for prochloraz-urea showed an increase of concentration over the 56 days duration of the experiments. In 1995, its final values reached 58 $\mu g kg^{-1}$ and 95 $\mu g kg^{-1}$ and in the following year they were 67 $\mu g kg^{-1}$ and 95 $\mu g kg^{-1}$ in NK1 and NW1, respectively. The results of all field experiments are depicted in Fig 3 to 6.

3.1.2 Laboratory experiments

Residue levels of prochloraz and its metabolites as well as mineralization and amounts of non-extractable residues after 56 days' incubation are compiled in Table 2 for all laboratory experiments conducted in the dark.

Prochloraz disappeared much more slowly under laboratory conditions. More than half of the prochloraz initially applied was still found after 56 days in a laboratory experiment with the soil NK1 incubated at 25 °C. Levels of prochloraz-formylurea varied, reaching a maximum of 5.2% after seven days,

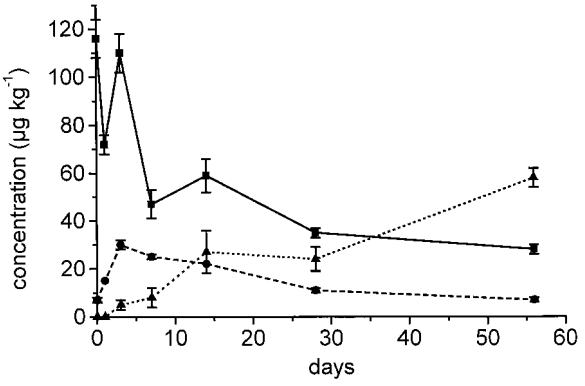

Figure 3. Concentrations in soil of (■) prochloraz, (●) prochloraz-formylurea and (▲) prochloraz-urea during the field experiment in Neuenkirchen, 1995 (samples analysed in duplicate).

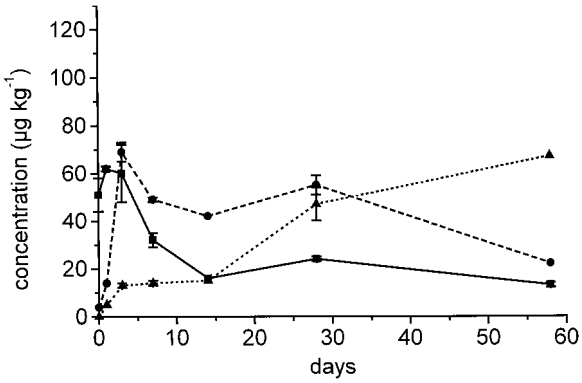

Figure 4. Concentrations in soil of (■) prochloraz, (●) prochloraz-formylurea and (▲) prochloraz-urea during the field experiment in Nienwohlde, 1995 (samples analysed in duplicate).

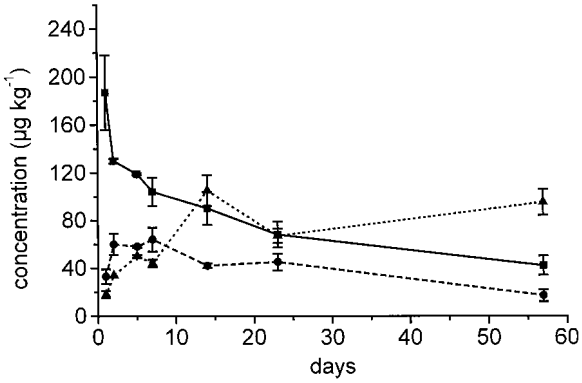

Figure 5. Concentrations in soil of (■) prochloraz, (●) prochloraz-formylurea and (▲) prochloraz-urea during the field experiment in Neuenkirchen, 1996 (samples analysed in duplicate).

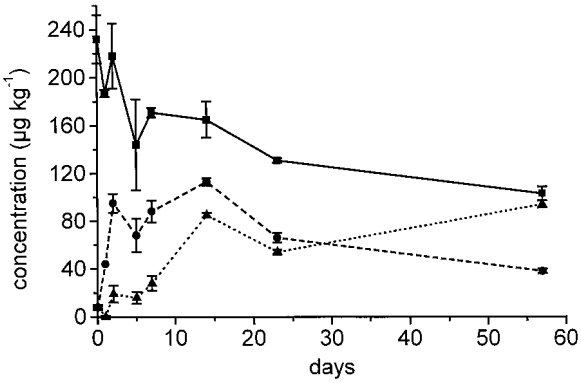

Figure 6. Concentrations in soil of (■) prochloraz, (●) prochloraz-formylurea and (▲) prochloraz-urea during the field experiment in Nienwohlde, 1996 (samples analysed in duplicate).

Soil	*Prochloraz*	*Prochloraz-formylurea*	*Prochloraz-urea*	*'Unkn1'*	*$^{14}CO_2$*	*Non-extr residues*
		(*% of initially applied radioactivity*)				
Soils from field experiments						
NK1[a]	64.4	3.4	3.7	2.6	6.4	14.1
NK1	38.3	n.d.[e]	1.5	4.5	6.4	26.6
NW1	47.7	n.d.[e]	0.9	10.8	0.8	30.2
Influence of organic carbon content						
NW2	56.1	0.4	1.8	2.4	3.2	17.4
NW3	60.1	0.3	1.2	10.8	1.1	16.6
NW4	58.4	n.d.[e]	1.1	10.8	1.4	17.7
Influence of soil texture						
NK2	39.3	0.2	1.9	1.3	16.4	28.4
NK1[b]	42.5	n.d.[e]	2.2	1.3	12.2	28.3
Influence of microbial activity						
NK1[c]	30.0	0.3	3.2	0.9	18.3	30.8
NK1[d]	57.2	0.5	0.8	1.4	0.1	21.5
NW1[d]	52.1	0.7	1.3	4.2	0.1	25.5

[a] Incubation at 25 °C, all other experiments at 30 °C.
[b] Control batch.
[c] Amended with 1% triticale straw.
[d] Sterilized.
[e] Not detected.

Table 2. Residue levels, mineralization ([^{14}C] carbon dioxide) and non-extractable residues after 56 days in laboratory experiments

whereas amounts of prochloraz-urea increased steadily, but did not exceed 3.7% after 56 days. In the further course of this experiment, prochloraz recovery gradually fell to 38% after 300 days. This loss was, however, not accompanied by an increase in metabolites. No prochloraz-formylurea was found at 112 and 300 days and amounts of prochloraz-urea remained constant at a level of about 3%. A mass balance of this experiment is given in Fig 7. In subsequent laboratory studies which were carried out at 30 °C, prochloraz disappeared distinctly faster in both soils. However, virtually no prochloraz-formylurea and only small amounts of prochloraz-urea were detected. Instead, an as yet unidentified polar metabolite ('unkn 1') was found, reaching high levels especially in NW1.

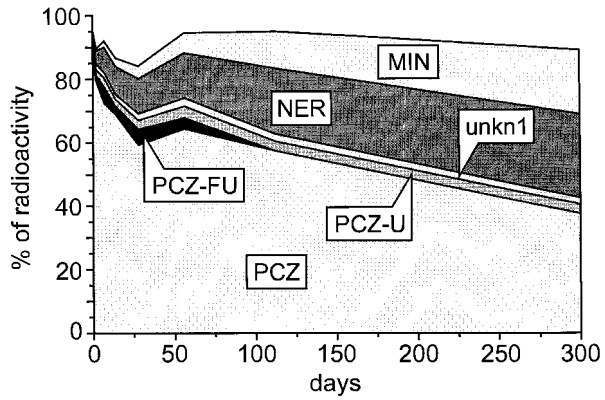

Figure 7. Amounts of prochloraz (PCZ), prochloraz-formylurea (PCZ-FU), prochloraz-urea (PCZ-U), an unknown polar metabolite (unkn1), non-extractable residues (NER) and mineralization (MIN) during a laboratory batch experiment on the clayey silt soil NK1 at 25 °C in the dark.

3.2 Prochloraz degradation as influenced by soil properties

3.2.1 Organic carbon content

The soils NW2, NW3, and NW4 originated from the same plot at Nienwohlde, but differed markedly in their organic carbon content. Residue dynamics in these soils were largely identical and showed similar tendencies to those observed for the soil NW1 (Table 2). For 'unkn 1', an increase of 5.5–5.9% was found within 28 days in NW2, NW3 and NW4. Thereafter, the amount of this compound dropped to 2.4% in NW2, as against the further increase seen in all other NW soils. Also, more mineralization occurred in NW2 as compared to NW1, NW3, and NW4, although the absolute amount of released [^{14}C]carbon dioxide was still low.

3.2.2 Soil texture

The soil NK2 differed from NK1 mainly in texture, having a higher amount of clay and a lower amount of silt. No significant differences in the amounts of parent compound and metabolites after 56 days' incubation were observed between NK2 and a control batch of NK1 which had been sampled at the same date (Table 2).

3.2.3 Microbial activity

Two approaches were followed to determine the impact of the soil's microbial activity on prochloraz degradation. Straw-amendment of the soil NK1 doubled the microbial activity (dehydrogenase activity determined by the triphenyl tetrazolium chloride method according to Malkomes[9] was 379 μg TPF g^{-1} $24h^{-1}$) as compared to a control sample (151 μg

Soil	Prochloraz	Prochloraz-formylurea	Prochloraz-urea	'Unkn1'	$^{14}CO_2$	Non-extr residues
		(% initially applied radioactivity)				
No irradiation (control)						
NK1	86.7	n.d.[a]	n.d.[a]	0.7	0.0	2.1
NW1	83.6	0.3	0.2	0.4	0.1	3.1
8h irradiation (4-h intervals)						
NK1	82.2	4.6	1.2	0.6	0.2	2.4
NW1	73.7	2.4	1.1	1.2	0.2	7.7
14h irradiation (15-min intervals)						
NK1	81.8	6.7	2.0	0.7	0.3	3.0
NW1	67.4	3.3	1.7	1.7	0.2	11.2
24h irradiation (15-min intervals)						
NK1	57.8	12.4	3.4	0.5	0.7	6.6
NK1[b]	68.8	6.0	1.3	0.2	0.2	4.1

[a] Not detected.
[b] Sterilized.

Table 3. Residue levels, mineralization ([^{14}C]carbon dioxide) and non-extractable residues in laboratory experiments under UV irradiation

TPF g^{-1} $24h^{-1}$). The amount of prochloraz in this soil decreased significantly faster and more prochloraz-urea was found than in most of the other laboratory experiments, although the amount of prochloraz-formylurea never exceeded 0.5%.

Concentrations of prochloraz also decreased after 56 days in degradation experiments carried out with sterilized soils NK1 and NW1. Small amounts of prochloraz-formylurea and prochloraz-urea were observed. In NW1, a significant amount of 'unkn 1' was also found.

3.3 Mineralization and non-extractable residues in laboratory studies

Regarding mineralization, three groups, can be distinguished in the laboratory studies. Virtually no mineralization was observed within the incubation period of 56 days with the sterilized soils NK1 and NW1. The amount of [^{14}C]carbon dioxide released in the soils from Nienwohlde was also very low even under non-sterile conditions, values ranging from 0.8% for NW1 to 3.2% for NW2. The highest mineralization rates (up to 18.3% in straw-amended soil NK1) were observed in the soils from Neuenkirchen.

Occurrence and amounts of non-extractable residues were not simply related to soil properties. At 30 °C, they amounted to about 30% in NK1, NK2 and NW1 after 56 days. Percentages in NW2, NW3, and NW4 were about half as high and did not vary with the organic carbon content. At 25 °C, the level of non-extractable residues in NK1 was only 14.1%. In the sterilized soils NK1 and NW1, amounts were slightly smaller than in the corresponding experiments under non-sterile conditions.

3.4 Impact of light on degradation of prochloraz

3.4.1 Residue dynamics

Residue levels for prochloraz and its metabolites, mineralization and amounts of non-extractable residues in the laboratory experiments under UV irradiation are presented in Table 3. Most obviously, concentrations of prochloraz-formylurea were distinctly higher under irradiation than in the dark. Increasing the irradiation time from 8h (in 4-h intervals) to 14h (in 15-min intervals) had no significant effect on the amount of prochloraz in NK1. Corresponding values for NW1 were lower and decreased with irradiation time. Nevertheless, more prochloraz-formylurea was formed in NK1 than in NW1. In two experiments with fresh and sterilized soil NK1, both irradiated for 24h (in 15-min intervals) within an incubation period of 66h, amounts of prochloraz were smaller and those of prochloraz-formylurea and prochloraz-urea were higher in the fresh than in the sterilized soil. The value of 12.4% for prochloraz-formylurea in non-sterilized soil NK1 was the highest amount of a metabolite found in any laboratory degradation experiment. Figure 8 shows the corresponding thin-layer chromatogram.

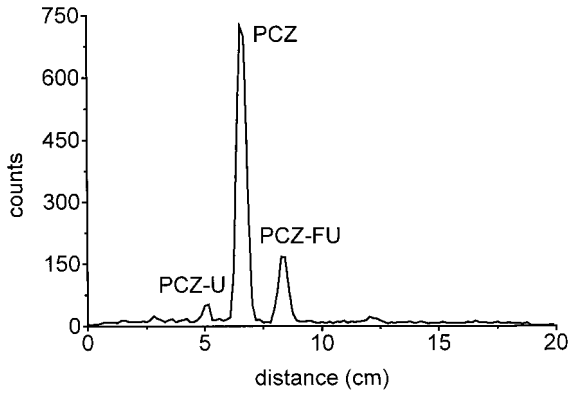

Figure 8. Thin-layer chromatogram of radioactive compounds in an extract from irradiated (24h) fresh clayey silt soil NK1, showing the presence of prochloraz (PCZ), prochloraz-formylurea (PCZ-FU) and prochloraz-urea (PCZ-U); development of M-N SILGUR 25 plate with toluene + ethanol, (15 + 1 by volume).

3.4.2 Mineralization and non-extractable residues

Mineralization was low in all irradiation experiments and typically did not exceed 0.3% in either soil, except for the fresh soil NK1 after 24 h irradiation where it amounted 0.7%.

As regards non-extractable residues, percentages in NW1 after 8 and 14 h irradiation time were about three times as high as in the corresponding experiments with the soil NK1. As with mineralization, the amount of non-extractable residues in the fresh soil NK1 after 24 h irradiation slightly surpassed the value found in sterilized soil NK1.

4 DISCUSSION AND CONCLUSIONS

Prochloraz half-lives of 11 to 43 days as found in the field studies agree well with values already published.[10] Persistence of this fungicide in topsoil (0–5 cm) may thus be assessed as low to intermediate. Together with the sorption behaviour, soil half-lives may be used for risk-assessment calculations, eg the GUS indices (groundwater ubiquity score) according to Gustafson.[16] Since prochloraz is sorbed strongly to soil with a K_{OC} of 12900,[17] these indices are virtually zero in all experiments, classifying prochloraz as an 'improbable leacher'.

In laboratory experiments as compared to field conditions, slower dissipation of prochloraz and distinct differences in metabolite formation were observed (Table 2). Since metabolite amounts were small even in soils with high microbial activity, other factors also seem to play a role in the degradation of prochloraz.

Prochloraz has been reported to undergo photolysis in aqueous solution, with a half-life of 10 days.[18] In sediment–water systems, the amount of prochloraz-formylurea formed under irradiation within 28 days was about ten times higher than in the dark.[4] In the present study, increased formation, particularly of the metabolite prochloraz-formylurea, occurred in irradiated batches only. However, microbial activity of the soil might have an impact on formation of this metabolite as well. Under identical irradiation conditions, twice the amount of prochloraz-formylurea was determined in fresh as compared to sterilized soil NK1 (Table 3). The assumption is also supported by a study on bacterial degradation of prochloraz that reported on formation of prochloraz-formylurea after incubating a prochloraz-containing medium with a strain of *Aureobacterium* sp.[5]

Photodegradation of pesticides on soil surfaces has been extensively studied, but most of the research has focused on purely abiotic processes.[19] However, in the case of prochloraz, both biotic and abiotic degradation seem to occur in the first step. Such a kind of 'photolytically activated microbial degradation' has already been suggested for the degradation of chloramben on soil under irradiation.[20]

Subsequent steps of prochloraz degradation, from the hydrolysis of prochloraz-formylurea to prochloraz-urea until mineralization, obviously depend on microbial metabolism. In the more acidic soils from Nienwohlde, formation of the as yet unidentified metabolite 'unkn 1' was promoted in competition with prochloraz-urea. Release of [^{14}C]carbon dioxide was strongly pH-dependent as well (Table 2).

Amounts of non-extractable residues in corresponding degradation experiments with fresh and sterilized soils (Table 2) suggest that their formation is based to a significant extent on abiotic processes. Despite the fact that sorption of prochloraz was found to depend primarily on soil organic matter content,[17] this was not the case for the non-extractable residues. Hence, the latter phenomenon seems to be based on soil–compound interactions different from those investigated in the sorption experiments.

Overall, the fate of prochloraz in soil is determined by a combination of photochemical and microbial degradation processes. Under irradiation, the parent compound is transformed into prochloraz-formylurea. Subsequently, this primary metabolite is hydrolysed to prochloraz-urea and finally mineralized. Since these microbial degradation steps depend on the previous photo-induced formation of prochloraz-formylurea, less degradation occurs in the dark. Consequently, consideration of such combined effects in the experimental design of degradation studies will enable a better comprehension of pesticide residue dynamics in soil.

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